DATA EVALUATION RECORD

FENAMIPHOS (NEMACUR®)

Study Type: §83-6; Developmental Neurotoxicity Study in Rats

Work Assignment No. 1-01-31 (MRID 46203401)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Michael E. Viana, Ph.D. Signature: _____
Date: _____

Secondary Reviewer:
David A. McEwen, B.S. Signature: _____

Date: _____

Program Manager:

Mary L. Menetrez, Ph.D. Signature: _____

Date: _____

Quality Assurance:

Steven Brecher, Ph.D. Signature: _____

Date: _____

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: John Liccione Ph.D Signature: _____

Toxicology Branch, Health Effects Division (7509C) Date _____

EPA Work Assignment Manager: P.V. Shah, Ph.D. Signature: _____

Registration Action Branch 1, Health Effects Division (7509C) Date _____

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 100601 DP BARCODE: D299719

TXR#: 0052413 SUBMISSION NO.: None

TEST MATERIAL (PURITY): Fenamiphos (Nemacur® technical; 95.6-95.9% a.i.)

SYNONYMS: Ethyl 3-methyl-4-(methylthio)phenyl(1-methylethyl) phosphoramidate

CITATION: Sheets, L.P. (2004) A developmental neurotoxicity screening study with technical grade fenamiphos (Nemacur®) in Wistar rats. Bayer CropScience LP, Toxicology, Stilwell, KS. Laboratory Study ID.: 02-D72-GP, January 26, 2004. MRID 46203401. Unpublished

SPONSOR: Bayer CropScience LP, 2 T. W. Alexander Drive, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46203401), fenamiphos (Nemacur®; 95.6-95.9% a.i., Batch # NCM-66) was administered to pregnant Wistar Hannover (Crl:WI [Glx/BRL/Han] IGS BR) rats (30/dose) in the diet at nominal dose levels of 0, 2.5, 10, or 50 ppm (equivalent to 0/0, 0.2/0.5, 0.9/2.1, and 4.8/10.3 mg/kg/day [gestation/lactation]) from gestation day (GD) 0 through lactation day (LD)

21. Animals presumed to be pregnant were subjected to a functional observational battery (FOB) on GD 6 and 20; a minimum of 10 dams/dose were also observed on LD 11 and 21. Dams were allowed to litter naturally and were killed on LD 21. On postnatal day (PND) 4, litters were standardized to 8 pups/litter (4 pups/sex as closely as possible); the remaining pups were killed and discarded without further examination. Subsequently, 1 pup/litter/group (at least 10 pups/sex/dose) were allocated to subsets for FOB, motor activity, acoustic startle habituation, learning and memory evaluations, ophthalmology, cholinesterase activity, gross pathology, and neuropathological examination. Acceptable positive control data from previously performed studies were obtained by the reviewers.

No treatment-related effects were seen on survival or reproductive performances. No treatment-related effects were observed on body weight or body weight gain during gestation. However, during lactation, body weight were decreased ($p < 0.05$) at the high dose (50 ppm) on LD 4-21. During lactation, food consumption was decreased ($p \leq 0.01$) by 10% in the 50 ppm on LD 14-21.
No effects on body weight, body weight gains, or food consumption were seen at the low or mid dose group dams. Plasma cholinesterase activity was decreased (37-85%) at ≥2.5 ppm. Erythrocyte cholinesterase activity was decreased ($p \leq 0.05$) by 61-85% at ≥10 ppm; and brain cholinesterase activity was decreased ($p \leq 0.05$) by 34% at 50 ppm.

The maternal LOAEL is 50 ppm (4.8 mg/kg/day) based on decreased body weights, body weight gains, and food consumption and increased incidence of tremors. The maternal NOAEL is 10 ppm (0.9 mg/kg/day).

The LOAEL for maternal cholinesterase inhibition is 10 ppm (0.9 mg/kg/day) based on inhibition of red blood cell cholinesterase activity. The NOAEL is 2.5 ppm (0.2 mg/kg/day).

Treatment had no adverse effects on offspring survival, clinical signs, FOB, learning and memory, brain weight, brain morphology or neuropathology. Decreased motor activity was seen in males only at the high dose on PND 13: 81% of the treated males in this group moved <40 time in any 10 minute interval compared to 63% of the PND 13 control males. A similar trend was not seen in females (treated,53% vs. control, 56%). In the PND 4 pups, plasma cholinesterase activity was decreased (19%, $p \leq 0.05$) at 50 ppm. At ≥10 ppm in the PND 21 pups, plasma cholinesterase activity was decreased ($p \leq 0.05$) by 21-67%, while erythrocyte and brain cholinesterase activities were decreased ($p \leq 0.05$) by 45-61% and 10-12%, respectively, at 50 ppm.

The offspring LOAEL is 50 ppm (4.8 mg/kg/day), based on decreased body weight and body weight gain, decrease in motor activity in males on PND 13 and inhibition of red blood cell and brain cholinesterase activity. The NOAEL is 10 ppm (0.9 mg/kg/day).

This study is classified Acceptable/Non Guideline and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive control data.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Fenamiphos (Nemacur®) technical

Description:	White solid
Lot/Batch #:	NCM-66
Purity:	95.6-95.9% a.i.
Compound Stability:	Stable in the diet for at least 7 days at room temperature or 28 days at freezer conditions
CAS # of TGAI:	22224-92-6
Structure:	

2. Vehicle and/or positive control: Diet

3. Test animals (P):

Species:	Rat
Strain:	Wistar Hannover (Crl:WI [Glx/BRL/Han] IGS BR)
Age at study initiation:	12 weeks
Weight at study initiation:	160.6-248.4 g
Source:	Charles River Laboratories (Raleigh, NC)
Housing:	Rats were housed individually in suspended stainless steel cages, except during cohabitation, when one male and one female were co-housed in the male's cage. During gestation and lactation, dams and their litters were housed in plastic cages with corn cob bedding. At weaning, litter mates were housed together in plastic cages with corn cob bedding; the following week, they were housed individually in suspended stainless steel cages.
Diet:	Purina Mills Rodent Lab Chow 5002 in meal form (Purina Mills Inc. Laboratories, St. Louis, MO), *ad libitum*, except during neurobehavioral testing
Water:	Tap water, *ad libitum,* except during neurobehavioral testing
Environmental conditions:	Temperature: 18-26°C Humidity: 30-70% Air changes: ≥10/hr Photoperiod: 12 hrs light/12 hrs dark
Acclimation period:	At least 6 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: April 29, 2002 End: August 1, 2002

2. Study schedule: The maternal animals were mated, assigned to study, and administered the test substance continuously in the diet from gestation day (GD) 0 through lactation day (LD) 21. On postnatal day (PND) 4, litters containing >8 pups were randomly standardized to 8 pups/litter (with equal pups/sex where ever possible) to reduce variability. Litters having <3 pups/sex, litters decreasing to 7 pups by PND 4, and dams without litters were killed and discarded without further examination. Pups were weaned on postnatal day 21, after which time maternal animals were killed. F_1 pups remained on study until PND 75 (study termination).

3. Mating procedure: Females were paired (1:1) with males of the same strain and source for a maximum of 4 consecutive days. Females and their cages were examined every morning for the presence of a copulatory plug, and vaginal smears were taken and examined for the presence of sperm. The day on which insemination was confirmed was designated as GD 0. After successful mating, females were placed into individual plastic nesting cages. Males were killed by CO_2 asphyxiation following mating and discarded.

4. Animal Assignment: After acclimation, females were weighed; any with body weights >±20% of the mean weight were rejected. The remaining females were assigned to the dose groups presented in Table 1 in sequence as they were determined to be inseminated.

Table 1. Study Design.[a]

Experimental Parameter	Dose (ppm)				
	0	2.5	10	50	Subset
Dams					
# of maternal animals	30	30	30	30	NA
FOB (GD 6 & 20)	30	30	30	30	NA
(LD 11 & 21)	10	10	10	10	NA
Cholinesterase determinations (LD 21)	10	10	10	10	NA
Offspring[b]					
Detailed clinical/FOB (PND 4, 11, 21, 35±1, 45±1, 60±2)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter	C
Motor activity (PND 13, 17, 21, and 60±2)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter	A
Acoustic startle habituation (PND 22, 38±2, 60±2)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter	B
Passive avoidance conditioning (PND 22, 29)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter	C
Water maze (PND 60±2, 67±2)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter	C

Experimental Parameter	Dose (ppm)				
	0	2.5	10	50	Subset
Ophthalmology (PND 50)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter	A, B, C
Cholinesterase determinations					
(PND 4)	10	10	10	10	A, B, C, D
(PND 21)	10/sex	10/sex	10/sex	10/sex	D
Brain weight, cerebrum and cerebellum					
length	10/sex	10/sex	10/sex	10/sex	D
(PND 21)	10/sex	10/sex	10/sex	10/sex	A, B, C
(PND 75±5)					
Neuropathology					
(PND 21)	10/sex	10/sex	10/sex	10/sex	D
(PND 75±5)	10/sex	10/sex	10/sex	10/sex	A, B, C

a Data were obtained from pages 27-28 and 36 of the study report.

b One male and/or one female per litter (approximately 16 [minimum 10]/sex/dose, representing at least 20 litters/dose).

NA Not applicable

5. Dose selection rationale: The doses presented in Table 1 were selected based on the results of a two-generation reproduction study (MRID 41908901) that was previously reviewed by the Agency (TXR 009473). In this study, fenamiphos was administered to Sprague Dawley rats via the diet at nominal doses of 0, 2.5, 10, or 40 ppm beginning approximately 10 weeks prior to mating. In the P generation females, plasma cholinesterase activity was decreased ($p \leq 0.05$) at ≥2.5 ppm (↓29-76%), erythrocyte cholinesterase activity was decreased ($p \leq 0.05$) at ≥10 ppm (↓39-51%), and brain cholinesterase activity was decreased ($p \leq 0.05$) at 40 ppm (↓21%). Additionally at 40 ppm, decreased body weight gains on LD 14 and 21 (↓8-9%) and decreased ovary weight (data not provided) were observed. In the F_1 generation on PND 21, plasma cholinesterase activity was decreased ($p \leq 0.05$) at ≥10 ppm in both sexes (↓26-66%), and erythrocyte cholinesterase activity was decreased ($p \leq 0.05$) at 40 ppm in both sexes (↓31-40%). Additional effects on the F_1 generation at 40 ppm included decreased body weights on PND 4-21 (↓6-17%), with lower body weights persisting until termination. In the F_2 generation on PND 4, plasma cholinesterase activity was decreased ($p \leq 0.05$) at 40 ppm (↓12%).

Based on these results, the doses selected for the developmental neurotoxicity study were 0, 2.5, 10, and 50 ppm. The 50 ppm dose was expected to provide clear evidence of toxicity in the dams, and measurable cholinesterase inhibition in the pups without excessive toxicity. The 2.5 ppm dose was expected to be the NOAEL dose in the pups and to cause a small inhibition of cholinesterase in the dams.

6. Dosage preparation administration and analysis: Dietary formulations were prepared weekly by dissolving appropriate amounts of the test compound in acetone and mixing with feed. The control diet was prepared the same way, excluding test compound. The formulations were stored at freezer conditions until presentation to the animals. The acetone was allowed to evaporate before diets were given to the animals. The dams were provided dietary formulations continuously beginning on GD 0 and continuing through LD 21. F_1 animals were not directly exposed to the test diets. Homogeneity (top, middle, and bottom assumed) and stability of the test compound in the diet were determined from samples of the 2.5 and 50 ppm dietary formulations prepared on Week 4. Stability was determined at room temperature for up to 7 days,

and at freezer conditions for up to 28 days. Concentration analyses were performed on all dietary formulations prepared on Weeks 1, 2, 3, and 6.

Results Homogeneity Analysis (% CV): 4.2-7.5%

Stability Analysis (range as % of initial):

After 7 days at room temperature: 101-102%

After 28 days at freezer conditions: 97.2-105%

Concentration Analysis (range as % of nominal): 102-104%

The analytical data indicated that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals: Dams were observed at least daily (daily on weekends and holidays) for mortality, morbidity, and clinical signs of toxicity. Detailed examinations were performed daily from GD 0 to LD 21. Body weights and food consumption were recorded on GD 0, 6, 13, and 20, and on LD 0, 7, 14, and 21. Additionally, dams were weighed on LD 4.

Dams were subjected to a modified functional observational battery (FOB), including examinations in the home cage, during handling, and in an open field, on GD 6 and 20 and on LD 11 and 21. The technicians were blind as to the dose group of the animal being tested. The FOB included, but was not limited to, the following.

FUNCTIONAL OBSERVATIONS	
X	Signs of autonomic function, including: 1) Lacrimation and salivation 2) Piloerection

	3) Exophthalmus 4) Urination and defecation 5) Pupillary function 6) Palpebral closure
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), and general signs of toxicity.

b. Offspring

1) Litter observations: As soon as possible following parturition, pups were weighed and sexed. All pups were observed at least daily for mortality, morbidity, and clinical signs of toxicity. Detailed observations were performed daily until weaning, then weekly thereafter. Pups were weighed on PND 0, 4 (pre- and post-culling), 11, 17, and 21, and weekly thereafter. Post-weaning food consumption was not recorded.

On PND 4, litters containing >8 pups were randomly standardized to 8 pups/litter (with equal sexes where possible) to reduce variability. Excess pups were used for cholinesterase activity determinations or killed and discarded. Litters with <3 pups/sex and litters that decreased to <7 pups by PND 4 were killed and discarded.

2) Developmental landmarks: All male pups were examined for preputial separation beginning on PND 38; all female pups were examined for vaginal patency beginning on PND 29. The day of onset and body weight on day of onset was recorded for each pup. On PND 21, all pups were tested for the presence of pupillary constriction by use of a penlight.

3) Postweaning observations: After weaning on PND 21, offspring were examined for mortality and morbidity daily. Detailed examinations and body weights were recorded weekly until study termination.

4) Neurobehavioral evaluations

i) Functional observational battery (FOB): The evaluation criteria for the modified FOB were not provided; however, scoring values were provided in the summary tables. On PNDs 4, 11, 21, 35±1, 45±1, and 60±2, subset C animals (1 pup/litter) were subjected to a modified FOB outside the home cage, as appropriate for the developmental stage being observed. The same parameters assessed in the maternal FOB were examined in the offspring. The technicians were blind as to the dose group of the animal being tested. The rats used in the FOB were also used in the passive avoidance conditioning and water maze tests.

ii) Motor activity testing: Motor activity was evaluated in subset A animals (1 pup/litter) on PNDs 13, 17, 21, and 60±2 in figure-eight mazes. Activity counts were recorded by a Universal Maze Monitoring System (Columbus Instruments, Columbus, OH) with a personal computer used for automated data collection. Data were collected in ten minute intervals over a 60 minute session. Motor activity was measured as the number of beam interruption occurring during the test session; locomotor activity was measured by eliminating consecutive counts for a given beam. Broad-spectrum background noise [74±2 dB (A)] was provided during testing to minimize acoustical variations. The uniformity of light intensity (100±70 Lux) over each maze was verified daily.

iii) Auditory startle reflex habituation: Auditory startle reflex habituation was evaluated in subset B animals (1 pup/litter) on PNDs 22, 38±2, and 60±2 using an integrated startle response test system (Coulbourn Instruments, Allentown, PA). Startle system enclosures were ventilated, lined with sound-attenuating and vibration-absorbing material, and contained 4 load cell/force transducer assemblies, allowing simultaneous testing of 4 rats in individual cages. Rats were exposed to a 50 millisecond burst (0 millisecond rise/fall) of broad-spectrum white noise at approximately 118 dB (lin). Test sessions consisted of 50 trials following a 5 minute adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus at 10 second intervals. Peak response amplitude (maximum value of the average curve minus baseline) and latency to peak were measured, and average response amplitude, latency to peak, and habituation over blocks of 10 trials were compared.

iv) Learning and memory testing: Learning and memory testing was performed on 1

set of subset C animals (1 pup/litter). On PND 22 and 29, learning, short-term retention, and long-term retention were tested in a passive avoidance test using an automated shuttle cage (Coulbourn Instruments) housed in an isolation cubicle. Shuttle cages consisted of 2 compartments of equal size; one compartment was lined with black film (dark side), the other was illuminated with a high-intensity lamp (lighted side). A centrally-located sliding (guillotine-type) door allowed access between the compartments. After an adaptation period (not described), rats were placed in the lighted side of the shuttle cage, facing the lamp. The lamp was illuminated (trial start) and the door was opened, allowing the rat access to the dark compartment. Movement of the rat into the dark compartment was detected by a photocell, causing the door to close, the lamp to turn off, and a brief (0.5 second) mild (0.5 mA) shock to be delivered through the grid floor of the cage. The rat was then placed in a holding cage until the next trial (time not specified). Rats that did not cross within 180 seconds were given a latency score of 180, and the procedure was repeated until either the rat remained in the lighted compartment for 180 seconds on 2 consecutive trials, or until 15 trials had been performed (learning phase). The test was repeated 1 week later (retention phase). Animals that failed to reach criterion performance within 15 trials or failed to cross during the first 2 trials were excluded from retention phase testing. Number of trials-to-criterion, latency to cross during learning and retention phases, and number of rats/group that failed to reach criterion within 15 trials (learning phase) were recorded.

Learning and memory were further tested on PND 60±2 and 1 week later in the same animals with a water maze. An M-maze filled with water to a depth of approximately 7.5 inches (22±1°C) was used to test acquisition and retention. The rat was placed in the maze at the base of the stem, located between the 2 lateral arms. On the first trial (learning trial), the rat was required to enter both arms of the maze before access to the exit ramp was provided. The initial arm chosen was designated as the incorrect goal during the subsequent trials (15 maximum). Rats that failed to make a correct goal choice within 60 seconds were guided to the correct arm and then removed from the water. Between trials, the rat was placed in a transport cage for 15±5 seconds. Rats were required to reach a criterion of 5 consecutive error-free trials to end the session. Latency to choose the correct goal and the number of errors/trial were

recorded. Animals attaining the above criteria were tested for retention 7 days after acquisition; animals failing to reach criterion were excluded from retention testing. The correct goal and criterion were the same for both phases of testing. The following measures were compared: for acquisition, the number of trials to criterion, average number of errors, and latency to reach the correct goal on Trial 2; and for retention, the number of trials-to-criterion, average number of errors, and latency to reach the correct goal on Trial 1.

5) Ophthalmology: Ophthalmic exams were conducted on subsets A, B, and C animals (1 pup/litter) at approximately PND 50. The exams were performed in a semi-darkened room. The pupillary reflex was tested using a penlight or transilluminator with a mydriatic agent. The conjunctiva, cornea, and lens were examined either before or after dilatation with a slit lamp microscope; the vitreous humor, retina, choroid, and optic disc were examined with an indirect ophthalmoscope equipped with a condensing lens after dilation.

6) Cholinesterase determination: Brain, erythrocyte, and plasma cholinesterase activities were measured in the dams on LD 21 and in the pups on PND 4 and 21. PND 4 pups (10/sex/dose) were selected from offspring that were culled on that day; blood samples from pups within a litter were collected by decapitation and pooled to provide an adequate sample volume. Ten dams/dose and 10 pups/sex/dose were selected on LD 21 and PND 21, respectively; blood samples were collected from the orbital plexus. Brains were collected to assay immediately following blood collection. Samples were analyzed using a modification of the method described by Ellman, where 6,6'-dithiodinicotinic acid was used as the coupling reagent and change in absorbance was measured at 340 nm.

2. Postmortem observations

a. Maternal animals: P-generational animals were killed by CO_2 asphyxiation. Dams were killed on LD 21 after weaning their litters. Females that mated successfully but did not litter were killed on GD 24 and discarded. Females that were found moribund were killed; females found moribund or dead were necropsied to determine the cause

of death. Tissues were collected from these animals at the discretion of the Study Director.

b. Offspring: Pups found moribund were killed and necropsied; tissues were collected at the discretion of the Study Director. Pups found dead were necropsied and discarded. Pups selected for culling on PND 4 not used for cholinesterase activity measurements were killed and discarded. Animals selected for perfusion or fresh brain weight measurements were necropsied, and gross lesions from neural and muscle tissues were collected for possible microscopic examination.

On PND 21, subset D animals (10 pups/sex/dose) were deeply anesthetized with an intraperitoneal injection of pentobarbital, flushed with phosphate-buffered sodium nitrite via the left ventricle, and then fixed *in situ* with phosphate-buffered 1.0% (w/v) gluteraldehyde/4.0% (w/v) EM-grade formaldehyde. The brain (with olfactory bulbs) was collected, weighed, measured (anterior-to-posterior length of the cerebrum and cerebellum), and post-fixed in 10% buffered formalin. The brain was then divided into 8 coronal sections, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Brain sections reserved for morphometric measurements (levels 3-5 and 7) were stained with luxol fast blue/cresyl violet. Microscopic examinations and morphometric measurements (consisting of determining the thickness of the frontal cortex, parietal cortex, corpus callosum, and hippocampal gyrus, the horizontal width of the caudate putamen, and the height of the cerebellum) were performed on sections from the control and 50 ppm groups.

At study termination (approximately PND 75), animals selected for fresh brain weight (10 pups/sex/dose) were killed by CO_2 asphyxiation. The brain was then removed, weighed, and discarded. Animals selected for perfusion (10 pups/sex/dose) were anesthetized and perfused as described above. The CHECKED (X) tissues listed below were removed from all animals and post-fixed in 10% buffered formalin.

CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM	
BRAIN		SCIATIC NERVE	
X	Olfactory bulbs	X	Sciatic nerve
X	Cerebral cortex		
X	Midbrain	OTHER	
X	Cerebellum	X	Sural nerve
X	Hippocampus	X	Tibial nerve
X	Medulla oblongata		Peroneal nerve
X	Basal ganglion	X	Lumbar dorsal root ganglion
X	Thalamus	X	Lumbar dorsal root fibers
X	Hypothalamus	X	Lumbar ventral root fibers
SPINAL CORD		X	Cervical dorsal root ganglion
X	Cervical swelling	X	Cervical dorsal root fibers
X	Thoracic swelling	X	Cervical ventral root fibers
X	Lumbar swelling		
X	Cauda equina		
OTHER			
X	Gasserian ganglion		
X	Optic nerves		
X	Eyes		
X	Skeletal muscle (gastrocnemius)		

The brain, spinal cord (cervical, thoracic, lumbar), cauda equina, eyes, optic nerves, and gastrocnemius muscle were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The brain was sectioned, stained, and morphometric measurements performed as described above. The cervical and lumbar dorsal root ganglia, dorsal and ventral root fibers, gasserian ganglia and peripheral nerves (sciatic, tibial, and sural) were embedded in glycol methacrylate, sectioned, and stained with a modified Lee's stain. Microscopic examinations and morphometric measurements were

performed on sections from the control and 50 ppm groups.

D. DATA ANALYSIS

1. Statistical analyses: Statistical evaluations were performed using either Instem Computer Systems, SAS, or TASC software. Significance was denoted at $p \leq 0.05$ ($p \leq 0.001$ for Bartlett's test). Data were subjected to the following statistical procedures.

Parameter	Statistical test
Maternal and pup body weight and food consumption, FOB continuous data, total session motor and locomotor activity data, and acoustic startle response peak amplitude data	Bartlett's test for equal variances; if variances equal, ANOVA followed by Dunnett's test if significant. If variances unequal, Kruskal-Wallis test followed by Mann-Whitney U test if significant
FOB categorical data	General Linear Modeling and Categorical Modeling procedures, using Dunnett's test and Analysis of Contrasts for *post-hoc* comparisons
Motor and locomotor interval data and acoustic startle response amplitude data for each block of 10 trials	Repeated-measures ANOVA, using both test interval and test occasion as repeated measures, followed by ANOVA for treatment by interval interaction and Dunnett's test if significant
Passive avoidance latency data	Wilcoxon's test for time-to-failure
Passive avoidance number of trials-to-criterion, and water maze number of trials-to-criterion and number of errors	Kruskal-Wallis test and Wilcoxon's test for acquisition phase; Fisher's Exact Test for retention phase
Water maze latency data	Univariate ANOVA followed by Dunnett's test if significant
Micropathology frequency data	Screened for potential effects, then evaluated with a Chi-Square procedure followed by a one-tailed Fisher's Exact Test if significant

The statistical analyses were considered appropriate

2. Indices: The following indices were calculated using the formulas below:

Mating index (%) = $\frac{\text{\# of inseminated females}}{\text{\# of females co-housed with males}} \times 100$

Fertility index (%) = $\frac{\text{\# of pregnant females}}{\text{\# of inseminated females}} \times 100$

Live birth index (%) = $\frac{\text{\# of live pups born per litter}}{\text{total \# of pups per litter x}} \times 100$

Viability index (%) = $\frac{\text{\# of live pups on PND 4 pre-culling per litter}}{\text{\# of live pups born per litter}} \times 100$

Lactation index (%) = $\frac{\text{\# of live pups on PND 21 per litter}}{\text{\# of live pups on PND 4 post-culling per litter}} \times 100$

3. Positive control data: Summaries of seven studies (MRIDs 45540501 through 45540507) performed to generate positive control data and validate the procedures and observers of the performing lab to conduct the FOB and to assess motor activity, neurotoxicity and behavioral effects were previously provided. These studies are under review.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality, clinical signs, and functional observations: All dams survived to scheduled termination. There were no clinical signs of toxicity observed during gestation. One 50 ppm dam was observed to have tremors during LD 17-21. During FOB testing on LD 21, two 50 ppm dams were observed to have urine stain, and one 50 ppm dam was

observed to have tremors. No other clinical signs of toxicity or FOB findings were observed.

2. Body weight and food consumption: Body weights and body weight gains for the dams are presented in Table 2. No effects of treatment were observed on body weight or body weight gains during gestation. However, during lactation, body weights were decreased ($p \leq 0.05$) by 6-15% in the 50 ppm females on LD 4-21. Overall (LD 0-21) body weight gains were decreased by 46% (calculated by reviewers). No effects on body weight or body weight gains were observed at 2.5 or 10 ppm.

Table 2. Mean (±SE) maternal body weights (g) of female rats administered fenamiphos in the diet from GD 0 to LD 21.[a]

Study Day	Dose (ppm)			
	0	2.5	10	50
Gestation (n=28-29)				
0	204.1±3.07	202.2±2.45	201.8±2.14	198.4±2.92
6	223.8±3.96	222.6±2.80	220.4±2.04	215.5±3.63
13	248.9±4.63	246.7±3.24	247.3±2.42	243.4±3.25
20	309.0±6.04	307.5±5.04	308.6±3.99	298.5±5.72
Overall body weight gain (GD 0-20)	104.8±4.16	105.3±3.33	106.8±2.86	100.1±4.09
Lactation (n=22-29)				
0	242.8±3.82	241.3±3.66	237.9±3.54	229.6±3.95
4	261.4±4.38	261.0±3.96	258.0±4.49	246.0±3.34* (↓6)
7	272.5±4.32	269.1±4.02	266.5±3.70	248.9±3.43** (↓9)
14	288.3±4.06	287.5±3.80	282.1±3.85	244.0±4.68** (↓15)
21	283.4±4.06	280.3±4.68	277.4±3.46	251.7±3.68** (↓11)
Overall body weight gain (LD 0-21)[b]	40.6	39.0	39.5	22.1 (↓46)

a Data were obtained from pages 62 and 68 of the study report. Percent differences from controls,

calculated by reviewers, are included in parentheses.

b Calculated by reviewers from data presented in this table

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

No effect of treatment was observed on food consumption during gestation. During lactation, food consumption was decreased ($p \leq 0.01$) by 10% in the 50 ppm females on LD 14-21 (Table 3). No effect on food consumption was observed at 2.5 or 10 ppm.

Table 3. Mean (±SE) maternal food consumption (g/day) of female rats administered fenamiphos in the diet from GD 0 to LD 21.[a]

Interval	Dose (ppm)			
	0	2.5	10	50
Gestation (n=27-29)				
GD 0-6	17.9±0.45	16.8±0.60	16.5±0.46	16.9±0.73
GD 6-13	20.3±0.79	19.5±0.63	19.9±0.42	21.8±0.90
GD 13-20	21.2±0.47	21.2±0.49	21.1±0.46	22.3±0.82
Lactation (n=21-23)				
LD 0-7	36.5±1.28	38.0±1.87	36.6±1.05	39.5±2.66
LD 7-14	52.2±0.96	53.4±1.14	54.4±1.20	49.0±1.62
LD 14-21	66.5±1.12	67.7±1.43	68.6±1.88	59.6±1.34** (↓10)

a Data were obtained from pages 64 and 70 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.

** Significantly different from controls; $p \leq 0.01$

3. Test Substance Intake: Mean compound intake (mg/kg bw/day) during gestation and lactation was calculated using the following relationship:

Average daily intake of active ingredient (a.i.) = [μg of a.i./g feed/1000]x[feed consumed (g/kg bw/day]

From these data, the mean intake was calculated for each of the 3 weeks of gestation and lactation (Table 4).

Table 4. Mean (±SE) test substance intake (mg/kg bw/day) in female rats administered fenamiphos in the diet from GD 0 to LD 21.[a]

Interval	Dose (ppm)[b]		
	2.5	10	50
Gestation (27-29)			
GD 0-6	0.2±0.01	0.8±0.02	4.4±0.19

GD 6-13	0.2±0.01	0.9±0.02	5.2±0.20
GD 13-20	0.2±0.00	0.9±0.02	4.7±0.17
Mean overall (GD 0-20) intake	0.2	0.9	4.8
Lactation (21-23)			
LD 0-7	0.4±0.02	1.6±0.05	8.6±0.54
LD 7-14	0.5±0.01	2.1±0.04	10.0±0.31
LD 14-21	0.6±0.01	2.5±0.08	12.3±0.26
Mean overall (LD 0-21) intake	0.5	2.1	10.3

a Data were obtained from pages 43 and 72-73 of the study report.

b The analytically determined concentrations of fenamiphos were 2.61, 10.2, and 51.1 ppm.

4. Reproductive performance: No effects of treatment were observed on reproductive performance at any dose (Table 5).

Table 5. Reproductive observations in female rats administered fenamiphos in the diet from GD 0 to LD 21.[a]

	Dose (ppm)			
Observation	0	2.5	10	50
Number mated	30	30	30	30
Number of litters	23	23	22	23
Mating index (%)	100	100	100	100
Fertility index (%)	96.7	93.3	93.3	96.7
Mean (±SE) gestation duration (days)	21.8±0.10	21.7±0.10	21.6±0.10	21.9±0.07

a Data were obtained from pages 58 and 75 of the study report.

5. Maternal postmortem results

a. Macroscopic examination: No macroscopic examinations were performed, as no dams were found dead or sacrificed in moribund condition.

b. Microscopic examination: No microscopic examinations were performed on the

dams.

c. Cholinesterase determinations: Maternal cholinesterase determinations are presented in Table 6. On LD 21, plasma cholinesterase activity was decreased ($p \leq 0.05$) by 37-85% at ≥2.5 ppm; erythrocyte cholinesterase activity was decreased ($p \leq 0.05$) by 61-85% at ≥10 ppm; and brain cholinesterase activity was decreased ($p \leq 0.05$) by 34% at 50 ppm.

Table 6. Mean (±SD) cholinesterase activity in female rats administered fenamiphos in the diet on GD 0 to LD 21.[a]

Compartment	Dose (ppm)			
	0	2.5	10	50
Plasma (IU/mL)	0.59±0.14	0.37±0.05* (↓37)	0.25±0.09* (↓58)	0.09±0.03* (↓85)
Erythrocyte (IU/mL)	1.24±0.20	1.06±0.26	0.48±0.14* (↓61)	0.19±0.14* (↓85)
Brain (IU/g)	12.8±0.5	12.5±0.5	12.3±0.9	8.5±0.5* (↓34)

a Data were obtained from page 868 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.

* Significantly different from controls; $p \leq 0.05$

B. OFFSPRING

1. Viability and clinical signs: F_1 litter size and viability data are presented in Table 7. No effects of treatment were observed on live litter size or post-natal survival through PND 21. Sex ratio (% male pups) was not provided. No treatment-related clinical signs of toxicity were observed.

Table 7. F_1 live litter size and viability.[a]

	Dose (ppm)			
Observation	0	2.5	10	50
# of litters	23	23	22	23
Total # of pups born	249	253	253	254
Total # of pups missing	2	2	1	2
Litters with pups missing	2	2	1	2
Total # of pups found dead	2	1	0	1
Litters with pups found dead	2	1	0	1
Total # of pups cannibalized	0	0	0	0
Total # of stillborn pups	0	0	0	0
Mean (±SE) litter size	10.8±0.31	11.0±0.38	11.5±0.41	11.0±0.38
Mean # of viable pups				
Day 0	11	11	12	11
Day 4[b]	11	11	11	11
Day 4[c]	8	8	8	8
Day 21	8	8	8	8
Sex ratio (% male)	NP	NP	NP	NP
Live birth index (mean±SE)	100±0.0	100±0.0	100±0.0	100±0.0
Viability index (mean±SE)	99.2±0.54	98.9±0.62	99.6±0.41	99.7±0.31
Lactation index (mean±SE)	99.5±0.54	100±0.0	100±0.0	98.9±0.75

a Data were obtained from pages 75-76 of the study report.

b Before standardization (culling).

c After standardization (culling).

NP Not provided

2. Body weight and food consumption: At 50 ppm, pre-weaning body weights were decreased ($p \leq 0.01$) by 13-18% in both sexes on PND 11-21 (Table 8a). Overall (PND 0-21) body weight gains were also decreased by 19-20% in both sexes (calculated by reviewers). Additionally at 50 ppm, decreased ($p \leq 0.05$) body weights persisted in the males (↓5-9%; Table 8b) throughout post-weaning (PND 29-71), and were observed in the females on PND 29-44 (↓4-7%). Decreased ($p \leq 0.05$) body weights were noted in the 10 ppm males on PND 43-71 (↓4%), but these findings were minor and considered incidental.

No treatment-related effect was observed on food consumption in the post-weaning offspring. Decreased ($p \leq 0.05$) food consumption was noted in the 10 ppm males (↓4%), but this finding was minor and considered incidental.

Table 8a. Mean (±SE) F_1 pup pre-weaning body weights and body weight gains (g).[a]

PND	Dose (ppm)			
	0	2.5	10	50
Males				
0	6.0±0.13	6.0±0.09	6.0±0.10	5.9±0.08
4[b]	10.3±0.23	9.9±0.23	9.9±0.27	9.7±0.18
4[c]	10.3±0.24	9.9±0.23	9.9±0.27	9.6±0.18
11	26.1±0.54	25.0±0.47	24.8±0.56	22.5±0.34** (↓14)
17	39.9±0.65	39.3±0.73	39.0±0.78	32.6±0.64** (↓18)
21	51.7±0.95	50.4±0.88	49.6±1.10	42.5±0.82** (↓18)
Overall (PND 0-21) gain[d]	45.7	44.4	43.6	36.6 (↓20)
Females				
0	5.7±0.10	5.6±0.09	5.7±0.12	5.6±0.09
4[b]	10.0±0.19	9.5±0.23	9.4±0.28	9.3±0.17

4[c]	10.0±0.19	9.5±0.24	9.4±0.28	9.3±0.17
11	25.2±0.45	24.2±0.52	23.9±0.54	21.9±0.35** (↓13)
17	38.4±0.57	38.1±0.70	37.4±0.81	31.6±0.62** (↓18)
21	49.7±0.80	48.6±0.94	47.4±0.99	41.2±0.76** (↓17)
Overall (PND 0-21)	44.0	43.0	41.7	35.6 (↓19)

a Data were obtained from pages 85-87 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses. n=22-23 litters (pre- and post-culling)

b Before standardization (culling).

c After standardization (culling).

d Calculated by reviewers from data presented in this table

** Significantly different from controls; $p<0.01$

Table 8b. Selected mean (±SD) F_1 pup post-weaning body weights and body weight gains (g).[a]

	Dose (ppm)			
PND	0	2.5	10	50
Males				
29	78.5±9.7	81.2±6.2	77.7±7.9	71.5±7.1* (↓9)
50	218.7±18.4	221.9±17.6	210.1±17.4* (↓4)	204.0±19.3* (↓7)
71	323.9±28.2	327.3±23.4	309.7±26.1* (↓4)	306.3±23.7* (↓5)
Overall (PND 29-71) gain[b]	245.4	246.1	232.0	234.8
Females				
30	78.0±8.1	80.1±7.6	76.8±6.1	72.7±6.3* (↓7)
44	140.8±11.0	142.4±11.3	138.3±10.3	135.0±8.5* (↓4)
72	198.3±18.8	199.7±16.4	198.0±47.6	193.8±13.6
Overall (PND 30-72)	120.3	119.6	121.2	121.1

a Data were obtained from pages 96-97 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.

b Calculated by reviewers from data presented in this table

* Significantly different from controls; $p<0.05$

3. Developmental landmarks

a) Sexual maturation: No effects of treatment were observed on either time to preputial separation or time to vaginal patency (Table 9).

Table 9. Mean (±SE) time (days) to sexual maturation in F_1 rats.[a]

	Dose (ppm)			
Parameter	0	2.5	10	50
# of litters	23	23	22	23
Preputial separation (males)	42.7±0.27	42.9±0.31	42.8±0.28	43.0±0.32

Vaginal opening (females)	33.0±0.26	33.5±0.27	33.2±0.33	33.3±0.25

a Data were obtained from page 94 of the study report.

b) Physical landmarks: No treatment-related effects were observed on pupil constriction.

4. Behavioral assessments

a) Functional observational battery: No treatment-related behavioral effects were observed at any dose in either sex.

b) Motor activity: No significant differences from controls were observed in either total session motor activity or total session locomotor activity in males and females at PND 17, 21, or 60 (Tables 10a and b). Motor activity was decreased in PND 13 males at 38%, 27%, and 54% and in PND 13 females at 44%, 57%, and 23% at the low, mid and high dose levels, respectively. Individual animal data that show the number of animals considered moving appropriately for their age (defined as >40 movements in any 10 minute block) are shown in Table 10c. On PND 13 at the high dose, 81% of treated males moved compared to 63% of control males; a similar trend was not seen among females.

Table 10a shows a decrease in motor activity in males at the high dose on PND 13, which is corroborated by the individual animal data in Table 10c. PND 13 females also showed a decrease in motor activity at the high dose (Table 10a); however, the data in Table 10c do not corroborate these findings. Therefore, a treatment-related effect in motor activity is seen only in males at the high dose on PND 13.

Table 10a. Mean (±S.D.) motor activity data (counts) in F_1 pups.[a]

PND	Dose (ppm)			
	0	2.5	10	50
Males				
13	90±81	56±51	66±65	41±45
17	169±103	165±154	212±118	182±76
21	344±134	346±78	341±134	355±125
60	536±91	532±138	516±117	571±116
Females				
13	90±63	50±66	39±40	69±46
17	131±105	151±108	131±130	195±140
21	344±122	361±118	306±124	341±90
60	658±122	741±200	637±138	722±113

a Data were obtained from pages 184-185 of the study report. n=15=16

Table 10b. Mean (±S.D.) locomotor activity data (counts) in F_1 pups.[a]

PND	Dose (ppm)			
	0	2.5	10	50
Males				
13	14±19	7±9	8±10	4±5
17	41±34	38±39	53±37	47±21
21	101±32	101±22	111±59	103±36
60	379±80	378±120	361±99	406±103
Females				
13	11±18	6±10	6±7	5±5
17	32±34	36±26	32±37	50±45
21	102±36	100±28	92±31	100±30
60	451±117	509±173	404±104	482±91

a Data were obtained from pages 187-188 of the study report. n=15=16

Table 10c . Number of F_1 pups that moved less than 40 times in any 10 minute interval/Total Tested.[a]

PND	Dose (ppm)			
	0	2.5	10	50
Males				
13	10/16 (63%)	11/16 (69%)	11/16 (69%)	13/16 (81%)
Females				
13	9/16 (56%)	13/16 (81%)	14/16 (88%)	9/17 (53%)

a Data were obtained from pages 187-188 of the study report. n=15=16

c) Auditory startle reflex habituation: No effects of treatment were observed on auditory startle response peak amplitude (Table 11a) or latency to peak (Table 11b). Habituation was unaffected by treatment.

Table 11a. Mean (±SD) auditory startle reflex peak amplitude (g) in F_1 rats.[a]

	Dose (ppm)

Observation[b]		0	2.5	10	50
Males					
PND 22	Block 1	48±21	46±17	47±17	45±17
	Block 2	44±17	47±21	50±23	43±21
	Block 3	44±21	48±21	44±22	36±16
	Block 4	44±21	47±23	40±17	34±16
	Block 5	42±24	45±16	38±16	33±16
PND 60	Block 1	255±215	265±183	190±137	192±132
	Block 2	194±159	188±148	164±88	138±89
	Block 3	162±119	130±79	107±64	115±69
	Block 4	149±105	130±82	108±64	127±73
	Block 5	113±61	127±74	100±48	103±43
Females					
PND 22	Block 1	50±23	50±25	45±14	47±21
	Block 2	43±19	50±18	45±19	42±18
	Block 3	42±21	43±19	40±16	41±20
	Block 4	44±22	42±16	37±15	37±18
	Block 5	39±19	40±17	34±13	35±15
PND 60	Block 1	122±137	104±72	75±35	124±51
	Block 2	111±132	93±75	58±39	91±44
	Block 3	85±71	72±47	56±28	91±35
	Block 4	85±62	69±42	51±25	79±29
	Block 5	58±26	61±35	44±19	76±15

a Data were obtained from pages 211, 213-214, and 216 the study report. n=15-16

b Block = 10 consecutive trials

Table 11b. Mean (±SD) auditory startle reflex latency to peak (msec) in F_1 rats.[a]

Observation[b]		Dose (ppm)			
		0	2.5	10	50
Males					
PND 22	Block 1	44±10	35±9	41±9	44±9
	Block 2	39±8	34±12	37±7	44±10
	Block 3	38±10	33±10	36±9	39±10
	Block 4	38±9	33±9	33±6	42±9
	Block 5	36±9	34±7	35±9	39±7
PND 60	Block 1	40±6	40±3	39±4	39±4
	Block 2	37±5	39±5	38±4	38±4
	Block 3	39±6	40±5	39±4	38±4
	Block 4	38±5	38±4	38±5	37±6
	Block 5	37±6	38±4	39±5	37±4
Females					
PND 22	Block 1	44±11	38±7	42±9	39±9
	Block 2	41±9	38±9	38±12	34±12
	Block 3	37±10	38±7	40±14	33±14
	Block 4	36±11	39±10	37±12	33±12
	Block 5	35±8	39±13	35±11	34±13
PND 60	Block 1	41±7	42±5	41±5	38±2
	Block 2	41±6	42±6	41±4	38±5
	Block 3	40±6	41±7	41±4	37±6
	Block 4	42±6	40±8	43±5	38±5
	Block 5	42±7	41±6	43±6	40±7

a Data were obtained from pages 211, 213-214, and 216 the study report. n=15-16

b Block = 10 consecutive trials

d) Learning and memory testing: No effects of treatment were observed on learning or memory in any treated group in either the passive avoidance test (Table 12a) or the water maze test (Table 12b). In the passive avoidance test, all groups showed

evidence of learning (the latency to cross was greater in Trial 2 than in Trial 1 on PND 22) and memory (the latency to cross was greater in Trial 1 and reduced number of trials-to-criterion on PND 29). In the water maze test, all groups showed evidence of learning (the duration of Trial 2 was less than the duration of Trial 1 on PND 60) and memory (the duration of Trial 1 on PND 67 was less than the duration of Trial 1 on PND 60 and reduced number of trials-to-criterion on PND 67).

Table 12a. Mean (±SD) passive avoidance performance in F_1 rats.[a]

Parameter		Dose (ppm)			
		0	2.5	10	50
Males					
Learning (PND 22)	Trials to criterion	3.3±0.6	3.3±1.0	3.3±0.8	3.1±0.3
	Latency Trial 1 (sec)	30.0±29.3	26.6±15.0	34.8±42.7	42.3±31.5
	Latency Trial 2 (sec)	170.3±27.0	169.5±41.9	179.2±3.3	178.6±5.5
	Failed to meet criterion	0	0	0	0
	Failed to cross during learning phase	0	0	1	0
Memory (PND 29)	Trials to criterion	2.0±0.0	2.5±1.0	2.3±0.8	2.3±0.6
	Latency trial 1 (sec)	180.0±0.0	174.2±16.4	169.1±42.1	163.5±46.8
	Latency Trial 2 (sec)	180.0±0.0	173.9±17.1	169.2±41.9	175.3±18.9
Females					
Learning (PND 22)	Trials to criterion	3.3±0.7	3.1±0.3	3.4±0.9	3.0±0.0
	Latency trial 1 (sec)	47.2±52.3	25.1±14.5	45.0±53.2	23.5±15.7
	Latency trial 2 (sec)	180.0±0.0	173.9±24.5	163.8±42.1	180.0±0.0
	Failed to meet criterion	0	0	0	0
	Failed to cross during learning phase	0	0	0	0
Memory (PND 29)	Trials to criterion	2.3±0.6	2.1±0.5	2.1±0.3	2.2±0.4
	Latency trial 1 (sec)	166.9±35.7	180.0±0.0	172.6±29.6	168.5±24.8
	Latency Trial 2 (sec)	179.9±0.3	174.8±21.0	180.0±0.0	180.0±0.0

a Data were obtained from pages 218-219 of the study report. n=16

Table 12b. Mean (±SD) water maze performance in F_1 rats.[a]

Parameter		Dose (ppm)			
		0	2.5	10	50
Males					
Learning (PND 60±2)	Trials to criterion	7.8±3.4	8.9±3.6	9.3±3.6	7.3±1.2
	Trial 1 - errors	0.6±0.8	0.9±1.2	0.4±0.6	1.2±1.2
	Trial 1 - duration (sec)	21.4±15.8	24.5±18.3	17.8±11.1	21.1±17.9
	Trial 2 - errors	0.3±0.6	0.7±0.9	1.6±1.8* (↑433)	0.8±1.0
	Trial 2 - duration (sec)	15.6±11.0	20.8±16.3	27.3±19.2	18.9±13.6
	Failed to meet criterion	1	3	3	0
Memory (PND 67±2)	Trials to criterion	5.7±1.3	6.1±1.5	5.7±0.9	5.8±1.4
	Trial 1 - errors	0.4±0.9	0.3±0.6	0.6±1.2	0.5±0.8
	Trial 1 - duration (sec)	10.3±9.2	7.6±5.3	10.9±8.0	14.9±13.1
	Trial 2 - errors	0.1±0.5	0.5±0.8	0.1±0.3	0.1±0.5
	Trial 2 - duration (sec)	4.3±3.4	7.0±5.2	4.3±1.5	5.2±5.6
Females					
Learning (PND 60±2)	Trials to criterion	7.2±2.9	7.4±2.7	7.0±2.4	6.3±1.3
	Trial 1 - errors	0.9±1.1	1.0±1.0	0.4±0.7	0.6±0.6
	Trial 1 - duration (sec)	20.5±13.5	19.9±15.3	16.9±13.6	14.4±8.0
	Trial 2 - errors	0.4±0.8	0.8±1.1	0.2±0.4	0.2±0.4
	Trial 2 - duration (sec)	11.1±9.7	14.6±14.1	11.8±6.5	10.8±4.3
	Failed to meet criterion	0	0	0	0
Memory	Trials to criterion	5.6±1.1	5.6±1.4	5.6±0.9	5.4±1.0

Parameter	Dose (ppm)			
	0	2.5	10	50
Trial 1 - errors	0.4±1.0	0.3±1.0	0.8±1.2	0.1±0.5
Trial 1 - duration (sec)	10.3±10.6	8.1±6.7	11.4±8.3	7.1±5.6
Trial 2 - errors	0.1±0.5	0.0±0.0	0.1±0.3	0.0±0.0
Trial 2 - duration (sec)	4.7±2.6	3.7±0.9	4.7±3.2	4.1±1.4

a Data were obtained from pages 221-222 of the study report. n=13-16

5. Ophthalmology: No effects of treatment were observed during the ophthalmological examinations.

6. Postmortem results

a) Brain weights and brain measurements: No treatment-related effects were observed on brain weight or brain measurements (Table 13). Increased ($p \leq 0.05$) relative (to body) brain weights were noted in the ≥10 ppm PND 21 females, in the 50 ppm PND 21 males, and in the PND 75 non-perfused males; however, these increases were considered to be due to the decreased body weights observed in these animals.

Table 13. Mean (±SD) absolute (g) and relative (to body, %) brain weight and brain measurements (mm) in F_1 rats.[a]

Parameter	Dose (ppm)			
	0	2.5	10	50
Males				
PND 21				
Terminal body weight (g)	48.4±3.0	48.6±5.6	49.6±5.5	41.4±5.3* (↓14)
Absolute brain weight (g)	1.409±0.035	1.421±0.048	1.513±0.084* (↑7)	1.383±0.071
Relative (to body) brain weight (%)	2.919±0.173	2.950±0.305	3.072±0.243	3.386±0.410* (↑16)
Cerebrum length (mm)	13.49±0.27	13.59±0.39	13.92±0.34* (↑3)	13.49±0.34
Cerebellum length (mm)	7.03±0.37	7.24±0.14	7.34±0.19	7.11±0.31
PND 75 perfused				
Terminal body weight (g)	329.2±34.0	330.7±27.0	307.7±16.6	315.3±22.6
Absolute brain weight (g)	1.847±0.095	1.861±0.053	1.789±0.048	1.835±0.083
Relative (to body) brain weight (%)	0.565±0.047	0.566±0.048	0.583±0.033	0.584±0.028
Cerebrum length (mm)	14.77±0.44	14.65±0.40	14.73±0.31	14.73±0.49
Cerebellum length (mm)	7.95±0.31	7.95±0.33	7.70±0.30	8.04±0.34
PND 75 non-perfused				
Terminal body weight (g)	338.5±30.7	332.2±14.6	310.0±38.4	309.0±25.9 (↓9)
Absolute brain weight (g)	1.974±0.108	1.916±0.109	1.930±0.102	1.978±0.104
Relative (to body) brain weight (%)	0.586±0.050	0.577±0.032	0.630±0.074	0.642±0.037* (↑10)
Females				
PND 21				
Terminal body weight (g)	50.4±4.3	49.6±5.6	45.8±4.7 (↓9)	40.0±4.4* (↓21)
Absolute brain weight (g)	1.380±0.041	1.409±0.068	1.381±0.071	1.326±0.068
Relative (to body) brain weight (%)	2.754±0.187	2.865±0.265	3.032±0.179* (↑10)	3.342±0.344* (↑21)
Cerebrum length (mm)	13.35±0.17	13.53±0.23	13.37±0.28	13.43±0.26
Cerebellum length (mm)	7.08±0.16	7.20±0.39	7.08±0.27	7.12±0.21

Parameter	Dose (ppm)			
	0	2.5	10	50
PND 75 perfused				
Terminal body weight (g)	201.2±16.9	206.3±16.4	192.2±19.6	196.7±13.4
Absolute brain weight (g)	1.720±0.069	1.703±0.070	1.692±0.090	1.680±0.059
Relative (to body) brain weight (%)	0.861±0.086	0.830±0.066	0.885±0.060	0.856±0.050
Cerebrum length (mm)	14.17±0.20	14.31±0.33	14.15±0.32	14.17±0.34
Cerebellum length (mm)	7.76±0.29	7.54±0.24	7.74±0.39	7.68±0.38
PND 75 non-perfused				
Terminal body weight (g)	205.9±16.6	199.1±10.6	191.2±16.3	200.1±13.4
Absolute brain weight (g)	1.867±0.071	1.839±0.086	1.839±0.111	1.824±0.070
Relative (to body) brain weight	0.910±0.059	0.925±0.048	0.965±0.058	0.914±0.053

a Data were obtained from pages 898-905 of the study report. n=10

* Significantly different from controls; $p<0.05$

b) Neuropathology

1) Macroscopic examination: No effects of treatment were observed during gross pathological examinations.

2) Microscopic examination: No treatment-related histopathological findings were observed in any group. Axonal degeneration was noted in the peripheral nerves of the PND 75 animals, but this finding occurred with equal frequency in the control and 50 ppm animals, and is commonly observed in adult rats. An increase ($p\leq0.05$) in the thickness of the parietal cortex was observed in the 50 ppm females, but this finding was minor and not corroborated by other pathological findings and was not considered to be treatment-related (Table 14).

Table 14. Mean morphometric measurements in F_1 rats.[a]

Parameter	Dose (ppm)

	Males		Females	
	0	50	0	50
PND 21				
Frontal cortex (thickness)	1.7042	1.6582	1.6385	1.6563
Parietal cortex (thickness)	1.8185	1.7519	1.6983	1.7608* (↑4)
Caudate putamen (horizontal width)	3.0215	2.9979	2.8796	2.9213
Corpus callosum (thickness)	0.5099	0.4247	0.4725	0.4341
Hippocampal gyrus (thickness)	1.4729	1.3915	1.3840	1.3820
Cerebellum height (cerebellum/pons)	4.0746	4.0356	4.0949	4.2852
PND 75				
Frontal cortex (thickness)	1.8024	1.7882	1.6966	1.6940
Parietal cortex (thickness)	1.8820	1.8263	1.7920	1.8143
Caudate putamen (horizontal width)	3.4606	3.3664	3.4097	3.4133
Corpus callosum (thickness)	0.6359	0.6686	0.6046	0.5657
Hippocampal gyrus (thickness)	1.6525	1.6653	1.5721	1.5835
Cerebellum height	4.1307	3.9525	4.0419	3.9595

a Data were obtained from pages 907-915 of the study report. n=10

* Significantly different from controls; $p<0.05$

c) Cholinesterase determinations: Offspring cholinesterase determinations are presented in Table 15. In the PND 4 pups, plasma cholinesterase activity was decreased ($p\leq0.05$) by 19% at 50 ppm. At ≥10 ppm in the PND 21 pups, plasma cholinesterase activity was decreased ($p\leq0.05$) by 21-67%, while erythrocyte and brain cholinesterase activities were decreased ($p\leq0.05$) by 45-61% and 10-12%, respectively, at 50 ppm.

Table 15. Mean (±SD) cholinesterase activity in F_1 rats.[a]

Compartment	Dose (ppm)			
	0	2.5	10	50
PND 4 (n=14-18)[b]				
Plasma (IU/mL)	0.64±0.07	0.59±0.06	0.60±0.06	0.52±0.07* (↓19)
Erythrocyte (IU/mL)	1.40±0.46	1.39±0.39	1.34±0.50	1.12±0.52
Brain (IU/g)	4.5±0.7	4.5±0.7	4.2±0.3	4.1±0.3
PND 21 males (n=6-9)				
Plasma (IU/mL)	0.57±0.07	0.55±0.08	0.45±0.08* (↓21)	0.19±0.07*(↓67)
Erythrocyte (IU/mL)	1.58±0.25	1.39±0.30	1.33±0.17	0.62±0.29* (↓61)
Brain (IU/g)	11.7±0.3	11.6±0.5	11.6±0.6	10.3±0.7* (↓12)
PND 21 females (n=5-9)				
Plasma (IU/mL)	0.59±0.09	0.55±0.08	0.45±0.10* (↓24)	0.21±0.09 (↓64)
Erythrocyte (IU/mL)	1.42±0.14	1.41±0.25	1.34±0.24	0.78±0.50* (↓45)
Brain (IU/g)	11.5±0.5	11.4±0.3	11.7±0.4	10.3±0.3* (↓10)

a Data were obtained from page 863 and 865-866 of the study report. Percent differences from controls, calculated by reviewers, are included in parentheses.

b Both sexes combined in summary table

* Significantly different from controls; $p \leq 0.05$

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: There were no effects on reproduction parameters at any dietary level. Maternal compound-related effects consisted of decreased body weight, body weight gain, and food consumption during lactation, and inhibition of plasma, erythrocyte, and brain cholinesterase activities. Offspring compound-related effects consisted of reduced body weights in both sexes during lactation and after weaning, with females recovering after weaning, and inhibition of plasma, erythrocyte, and brain cholinesterase activity. The maternal NOAEL was 2.5 ppm (0.2/0.5 mg/kg/day); the offspring NOAEL was 10 ppm (0.9/2.1 mg/kg/day).

B. REVIEWER COMMENTS: .

No treatment-related effects were seen on survival or reproductive performances. No treatment-related effects were observed on body weight or body weight gain during gestation. However, during lactation, body weight were decreased ($p < 0.05$) at the high dose (50 ppm) on LD 4-21. During lactation, food consumption was decreased ($p \leq 0.01$) by 10% in the 50 ppm on LD 14-21.
No effects on body weight, body weight gains, or food consumption were seen at the low or mid dose group dams. Plasma cholinesterase activity was decreased (37-85%) at ≥2.5 ppm. Erythrocyte cholinesterase activity was decreased ($p \leq 0.05$) by 61-85% at ≥10 ppm; and brain cholinesterase activity was decreased ($p \leq 0.05$) by 34% at 50 ppm.

Treatment had no adverse effects on offspring survival, clinical signs, FOB, learning and memory, brain weight, brain morphology or neuropathology. Decreased motor activity was seen in males only at the high dose on PND 13: 81% of the treated males in this group moved <40 time in any 10 minute interval compared to 63% of the PND 13 control males. A similar trend was not seen in females (treated,53% vs. control, 56%). In the PND 4 pups, plasma cholinesterase activity was decreased (19%, $p \leq 0.05$) at 50 ppm. At ≥10 ppm in the PND 21 pups, plasma cholinesterase activity was decreased ($p \leq 0.05$) by 21-67%, while erythrocyte and brain cholinesterase activities were decreased ($p \leq 0.05$) by 45-61% and 10-12%, respectively, at 50 ppm.

The maternal LOAEL is 50 ppm (4.8 mg/kg/day) based on decreased body weights, body weight gains, and food consumption and increased incidence of tremors. The maternal NOAEL is 10 ppm (0.9 mg/kg/day).

The LOAEL for maternal cholinesterase inhibition is 10 ppm (0.9 mg/kg/day) based on inhibition of red blood cell cholinesterase activity. The NOAEL is 2.5 ppm (0.2 mg/kg/day).

The offspring LOAEL is 50 ppm (4.8 mg/kg/day), based on decreased body weight and

body weight gain, decrease in motor activity in males on PND 13 and inhibition of red blood cell and brain cholinesterase activity. The NOAEL is 10 ppm (0.9 mg/kg/day).

This study is classified Acceptable/Non Guideline and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive control data.

C. STUDY DEFICIENCIES: The following minor deficiencies were noted but do not change the conclusions of this review:

- The evaluation and scoring criteria for the functional observational battery were not provided.
- Sex ratio (% males) of the offspring was not reported.

D. NOTES

Regarding the memorandum dated September 20, 2000, the following points are noted:

3. The dosing period was increased to GD 0 through LD 21 as suggested.

4. Measurements of the test compound and/or metabolites in the blood and milk were not performed as recommended.

5. Cholinesterase activity measurements were determined in the dams on LD 21 and in the pups on PND 4 and 21. Measurements were not performed on GD 20 or during mid-lactation, as recommended. Pup blood samples were pooled by litter as suggested. Peripheral nervous system tissues were not tested for cholinesterase inhibition as recommended.

6. The number of pups selected for neuropathological examination was increased to 10 pups/sex/dose, and all nervous system tissues were fixed *in situ* as specified. Morphometric measurements were performed.

7. Ophthalmological examinations were performed on pups on PND 50; electroretinography was not performed as recommended.

8. Rationale for the selection of the doses was discussed in a memorandum dated April, 4, 2002 (TXR 0050621)